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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Mathur, et al. Art Unit : 1652
Serial No. : 09/202,681 Examiner : Richard Hutson, Ph.D.
Filed : December 23, 1999
Title : PHOSPHATASES, POLYNUCLEOTIDES ENCODING THEM AND METHODS OF MAKING AND USING THEM (amended)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, Jay Short, am an expert in the field of molecular biology and enzyme development and was an expert at the time of the invention. I am presently employed as CEO and as a research scientist at Diversa Corporation, San Diego, CA, assignee of the above-referenced patent application. My resume is attached as documentation of my credentials.

2. I declare that procedures for identifying nucleic acids that encode enzymes such as phosphatases were conventional and routine in the art at the time of the invention. Procedures for identifying polypeptides having phosphatase activity were conventional and routine in the art at the time of the invention. For example, an exemplary assay for identifying polypeptides having phosphatase activity is described in the paragraph spanning pages 39 and 40 of the WO 97/48416 specification. One of ordinary skill in the art using the teaching of the specification could have made and expressed nucleic acids having a percent sequence identity to an exemplary nucleic acid, or, which hybridized under defined conditions to an exemplary nucleic acid, and using routine screening could have determined with predicable positive results which of those nucleic acids encoded a polypeptide having phosphatase activity. Thus, using the teaching of the specification one of ordinary skill in the art would be able to ascertain the scope of the claimed genus of phosphatases and phosphatase-encoding nucleic acids with reasonable clarity and recognize that Applicants' were in possession of the claimed invention at the time of filing.

3. I declare that one of ordinary skill in the art using the teaching of the specification would have been able to make nucleic acids that encode for fragments of the exemplary phosphatase-encoding nucleic acid and express those nucleic acids, and using routine screening determine with predicable positive results which of the identified nucleic acids encode polypeptides having phosphatase activity. One of ordinary skill in the art using the teaching of the specification would have been able to ascertain what fragments of the exemplary nucleic acid could be used to identify phosphatase-encoding nucleic acids. One of ordinary skill in the art using the teaching of the specification would have been able to ascertain what fragments of the exemplary phosphatase could be used to identify enzymatically active fragments. Thus, using the teaching of the specification one of ordinary skill in the art would be able to ascertain the scope of the claimed genus of polypeptides with phosphatase activity and phosphatase-identifying nucleic acids with reasonable clarity and recognize that Applicants' were in possession of the claimed invention at the time of filing.

4. I declare that procedures for modifying and expressing nucleic acids were conventional and routine in the art at the time of the invention. Procedures for determining the activity of the expressed modified nucleic acids and determining if the nucleic acids expressed a polypeptide with phosphatase activity were conventional and routine in the art at the time of the invention. Procedures for determining sequence identity to an exemplary nucleic acid or whether a nucleic acid hybridized to a target nucleic acid under defined conditions were routine in the art at the time of the invention. Procedures for expressing and screening for phosphatase activity were conventional and routine in the art at the time of the invention.

5. I declare that one of ordinary skill in the art using the teaching of the specification would have been able to make phosphatase-encoding nucleic acids having at least 70% sequence identity to the exemplary nucleic acid, or phosphatase-encoding nucleic acids that hybridize under defined hybridization conditions to the exemplary nucleic acid, to make and use the genus of compositions of the invention without undue experimentation. It was considered routine by one skilled in the art at the time of the invention to screen for multiple substitutions or modifications of a nucleic acid or a polypeptide for functional variations, including screening for

a genus of phosphatase-encoding nucleic acids or a genus of phosphatases. It was considered routine by one skilled in the art at the time of the invention to screen for subsequences of nucleic acids that encode enzymatically active fragments of an exemplary enzyme. It was considered routine by one skilled in the art at the time of the invention to screen for subsequences of nucleic acids that can identify by hybridization a phosphatase-encoding nucleic acid. For example, high through-put methods for screening for enzyme activity, such as phosphatase activity, were well known in the art. While the numbers of samples needed to be screened may have been high, the screening procedures were routine and successful results (e.g., finding a genus of nucleic acids encoding phosphatases) predictable. At the time of the invention it would have been considered routine by one skilled in the art to generate and screen multiple substitutions or multiple modifications in an exemplary nucleic acid sequence and predictably generate a genus of phosphatase-encoding nucleic acids or a genus of phosphatases.

6. I declare that it was not necessary for the skilled artisan to understand which specific regions of phosphatase sequence or structure needed to be modified without affecting function or activity to routinely generate the claimed genus of phosphatase-encoding nucleic acids. Methods for sequence modifications were sufficiently comprehensive, routine and predictable at the time of the invention to predictably generate phosphatase-encoding sequences without need of knowing which specific regions of phosphatase sequence or structure affected phosphatase function or activity. Methods known at the time of the invention for modifying nucleic acid sequences in combination with high through-put enzyme activity screening known at the time of the invention, made methods that require previous knowledge of protein structure, including secondary or tertiary structure, active site sequences, and the like obsolete and unnecessary. Accordingly, using methods known in the art at the time of the invention it would not have been necessary to understand which specific regions of phosphatase structure needed to be modified to generate a genus of nucleic acids or polypeptides for practicing the invention without undue experimentation.

7. I declare that the specification provides sufficient guidance to one of ordinary skill in the art as to whether a nucleic acid or polypeptide falls within the scope of the claimed

genus. Methods for determining the requisite structure (sequence based on percent sequence identity to an exemplary nucleic acid or polypeptide) and function (phosphatase activity) are clearly set forth in the specification. At the time of the invention, high through-put *in vivo* (e.g., whole cell) nucleic acid expression and enzyme activity screening protocols were well known in the art. The specification sets forth an exemplary phosphatase screening assay to determine if a nucleic acid or polypeptide is within the scope of the claimed genus, inter alia, in the paragraph spanning pages 39 and 40 of the WO 97/48416 specification. Methods for determining sequence identity were also routine and well known in the art at the time of the invention. While the numbers of alternative species that needed to be screened may have been high, the protocols for screening were routine and positive results predictable. Accordingly, the specification provided sufficient guidance to one of ordinary skill in the art to make and use the claimed genus of nucleic acids or polypeptides to practice the invention.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted

Date: 5/19/04

Jay Short